

SEQUENCE LISTING

RECEIVED

APR 15 2002

TECH CENTER 1600/2900

B1

<110> YOKOYAMA, SIGEYUKI
HOSEKI, JUN
YANO, TAKATO
KOYAMA, YOSHINORI
KURAMITSU, SEIKI
KAGAMIYAMA, HIROYUKI

<120> MUTANT KANAMYCIN NUCLEOTIDYLTRANSFERASE AND A METHOD
OF SCREENING THERMOPHILIC BACTERIA USING THE SAME

<130> 04853.0048-00000

<140> 09/697,186

<151> 2000-10-27

<150> JP 309616/1999

<151> 1999-10-29

<160> 20

<170> PatentIn Ver. 2.1

<210> 1

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
obtained by introduction of point mutation into
wild type KNT gene of Staphylococcus aureus and
its expression

<400> 1

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe
50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Tyr
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp
85 90 95

Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
100 105 110



Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125
 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190
 Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
 195 200 205
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 2

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
 obtained by introduction of point mutation into
 wild type KNT gene of Staphylococcus aureus and
 its expression

<400> 2

Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15
 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30
 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45
 Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
 50 55 60
 Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr
 65 70 75 80
 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu
195 200 205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
245 250

<210> 3
<211> 253
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mutant enzyme
obtained by introduction of point mutation into
wild type KNT gene of Staphylococcus aureus and
its expression

<400> 3
Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe
50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp
 85 90 95
 Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
 100 105 110
 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125
 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190
 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Pro Gly Tyr Val Gln Leu
 195 200 205
 Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Pro Glu Lys Leu Leu
 210 215 220
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg
 225 230 235 240
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 4
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer for
 PCR amplification

<400> 4
 gactgtacgg gtacccgttg acggcggata tggta

35

<210> 5
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer for
 PCR amplification

<400> 5
 gactgtacgc tgcagcgtaa ccaacatgat taaca

35

<210> 6
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer for
PCR amplification

<400> 6
gactgtacgg aattcgagct cgagcaaatc taaaa 35

<210> 7
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer for
subcloning of WT

<400> 7
gactgtacgc atatgaatgg accaataata atgac 35

<210> 8
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer for
subcloning of KT3-11 and HTK

<400> 8
gactgtacgc atatgaaagg accaataata atgac 35

<210> 9
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer for subcloning

<400> 9
gactgtacgc tcgagcgtaa ccaacatgat taaca 35

<210> 10
<211> 759
<212> DNA
<213> Staphylococcus aureus

<220>

<221> CDS

<222> (1) .. (759)

<400> 10

| | |
|---|-----|
| gtg aat gga cca ata ata atg act aga gaa gaa aga atg aag att gtt | 48 |
| Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val | |
| 1 5 10 15 | |
| cat gaa att aag gaa cga ata ttg gat aaa tat ggg gat gat gtt aag | 96 |
| His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys | |
| 20 25 30 | |
| gct att ggt gtt tat ggc tct ctt ggt cgt cag act gat ggg ccc tat | 144 |
| Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr | |
| 35 40 45 | |
| tcg gat att gag atg atg tgt gtc atg tca aca gag gaa gca gag ttc | 192 |
| Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe | |
| 50 55 60 | |
| agc cat gaa tgg aca acc ggt gag tgg aag gtg gaa gtg aat ttt gat | 240 |
| Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp | |
| 65 70 75 80 | |
| agc gaa gag att cta cta gat tat gca tct cag gtg gaa tca gat tgg | 288 |
| Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp | |
| 85 90 95 | |
| ccg ctt aca cat ggt caa ttt ttc tct att ttg ccg att tat gat tca | 336 |
| Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser | |
| 100 105 110 | |
| ggt gga tac tta gag aaa gtg tat caa act gct aaa tcg gta gaa gcc | 384 |
| Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala | |
| 115 120 125 | |
| caa acg ttc cac gat gcg att tgt gcc ctt atc gta gaa gag ctg ttt | 432 |
| Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe | |
| 130 135 140 | |
| gaa tat gca ggc aaa tgg cgt aat att cgt gtg caa gga ccg aca aca | 480 |
| Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr | |
| 145 150 155 160 | |
| ttt cta cca tcc ttg act gta cag gta gca atg gca ggt gcc atg ttg | 528 |
| Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu | |
| 165 170 175 | |
| att ggt ctg cat cat cgc atc tgt tat acg acg agc gct tcg gtc tta | 576 |
| Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu | |
| 180 185 190 | |
| act gaa gca gtt aag caa tca gat ctt cct tca ggt tat gac cat ctg | 624 |
| Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu | |
| 195 200 205 | |

tgc cag ttc gta atg tct ggt caa ctt tcc gac tct gag aaa ctt ctg 672
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220

gaa tcg cta gag aat ttc tgg aat ggg att cag gag tgg aca gaa cga 720
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240

cac gga tat ata gtg gat gtg tca aaa cgc ata cca ttt 759
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 11
 <211> 253
 <212> PRT
 <213> Staphylococcus aureus

<400> 11
 Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe
 50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp
 85 90 95

Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
 100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 12

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
 obtained by introduction of point mutation into
 wild type KNT gene of Staphylococcus aureus and
 its expression

<400> 12

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15

Tyr Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe
 50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190

Thr Glu Ala Leu Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
245 250

<210> 13

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
obtained by introduction of point mutation into
wild type KNT gene of Staphylococcus aureus and
its expression

<400> 13

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asn Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe
50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Pro Asp Trp
85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
100 105 110

Gly Gly Tyr Leu Gly Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Leu Val Leu
 180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
 195 200 205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 14

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
 obtained by introduction of point mutation into
 wild type KNT gene of Staphylococcus aureus and
 its expression

<400> 14

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
 100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160

```

<400> 15
Met Ser Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
  1                               5                10                15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
                20                        25                30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
                35                        40                45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
  50                               55                60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
  65                        70                75                80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp
                85                        90                95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
                100                       105                110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
  115                        120                125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
  130                        135                140

```

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Thr Val Leu
 180 185 190
 Thr Glu Ala Val Lys Leu Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
 195 200 205
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Thr Glu Arg
 225 230 235 240
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 16
 <211> 253
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Mutant enzyme
 obtained by introduction of point mutation into
 wild type KNT gene of Staphylococcus aureus and
 its expression

<400> 16
 Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15
 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30
 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45
 Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Thr Glu Phe
 50 55 60
 Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
 65 70 75 80
 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp
 85 90 95
 Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Thr
 100 105 110
 Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Leu Thr
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Gly Ala Ser Val Leu
 180 185 190

Thr Glu Ala Val Arg Gln Pro Asp Leu Pro Pro Gly Tyr Asp His Leu
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Ala Glu Arg
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 17

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
 obtained by introduction of point mutation into
 wild type KNT gene of Staphylococcus aureus and
 its expression

<400> 17

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 18

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
 obtained by introduction of point mutation into
 wild type KNT gene of Staphylococcus aureus and
 its expression

<400> 18

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
 100 105 110
 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125
 Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190
 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu
 195 200 205
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 19

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme
 obtained by introduction of point mutation into
 wild type KNT gene of Staphylococcus aureus and
 its expression

<400> 19

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15
 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 20 25 30
 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 35 40 45
 Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
 50 55 60
 Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
 65 70 75 80

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ser | Glu | Glu | Ile | Leu | Leu | Asp | Tyr | Ala | Ser | Arg | Val | Glu | Pro | Asp | Trp | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| Pro | Leu | Thr | His | Gly | Lys | Phe | Phe | Ser | Ile | Leu | Pro | Ile | Tyr | Asp | Pro | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| Gly | Gly | Tyr | Leu | Glu | Lys | Val | Tyr | Gln | Thr | Ala | Lys | Ser | Val | Glu | Ala | |
| | | 115 | | | | | 120 | | | | | 125 | | | | |
| Gln | Thr | Phe | His | Asp | Ala | Ile | Cys | Ala | Leu | Ile | Val | Glu | Glu | Leu | Phe | |
| | 130 | | | | | 135 | | | | | 140 | | | | | |
| Glu | Tyr | Ala | Gly | Lys | Trp | Arg | Asn | Ile | Arg | Val | Gln | Gly | Pro | Thr | Thr | |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| Phe | Leu | Pro | Ser | Leu | Thr | Val | Gln | Val | Ala | Met | Ala | Gly | Ala | Met | Leu | |
| | | | | 165 | | | | | 170 | | | | | 175 | | |
| Ile | Gly | Leu | His | His | Arg | Ile | Cys | Tyr | Thr | Thr | Ser | Ala | Ser | Val | Leu | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| Thr | Glu | Ala | Val | Lys | Gln | Pro | Asp | Leu | Pro | Ser | Gly | Tyr | Val | Gln | Leu | |
| | | 195 | | | | | 200 | | | | | 205 | | | | |
| Cys | Gln | Phe | Val | Met | Ser | Gly | Gln | Leu | Ser | Asp | Pro | Glu | Lys | Leu | Leu | |
| | 210 | | | | | 215 | | | | | 220 | | | | | |
| Glu | Ser | Leu | Glu | Asn | Phe | Trp | Asn | Gly | Ile | Gln | Glu | Trp | Thr | Glu | Arg | |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 | |
| His | Gly | Tyr | Ile | Val | Asp | Val | Ser | Lys | Arg | Ile | Pro | Phe | | | | |
| | | | | 245 | | | | | 250 | | | | | | | |

<210> 20

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 20

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Glu Ala Glu Phe
50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp
 65 70 75 80
 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp
 85 90 95
 Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
 100 105 110
 Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125
 Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190
 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu
 195 200 205
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

end

B1